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Effects of Tetrabenazine on Methamphetamine-Induced Hyperactivity in Mice are Dependent on Order and Time-Course of Administration

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KURIBARA, H. *Effects of tetrabenazine on methamphetamine-induced hyperactivity in mice are dependent on order and time-course of administration.* PHARMACOL BIOCHEM BEHAV **56**(1) 9–14, 1997.—The ambulation-increasing effect of methamphetamine (MAP: 2 mg/kg SC) in mice persisted for about 3 h. Tetrabenazine (TBZ: 4 mg/kg SC), a depleter of monoamines from the cytoplasmic pool did not increase ambulation on its own. Pretreatment with TBZ at 1.5 h before administration of MAP inhibited the stimulant effect of MAP. In contrast, combined administration of two drugs resulted in a transient but considerable enhancement of MAPs stimulant effect. Post-MAP treatment with TBZ at 0.5–2 h hardly modified MAPs behavioral effects. In contrast, 3–6 h post-MAP treatment with TBZ induced a transient increase in activity, although the stimulant effect of MAP had already disappeared. The maximum increase in ambulatory stimulation was produced by 4-h post-MAP treatment with TBZ. The inhibitory effect of TBZ pretreatment on MAP-induced hyperactivity, as well as the transient hyperactivity elicited by TBZ when administered along with MAP, or 4 h after MAP, was dose-dependent. Preliminary studies revealed that transient hyperactivity was never produced by combination of GBR-12909 (a selective dopamine reuptake inhibitor) with TBZ or MAP with oxypertine (a selective norepinephrine releaser/depleter), but produced by combination of nialamide (a monoamine oxidase inhibitor) with TBZ. Inhibition of MAPs effects by TBZ pretreatment suggests that enhancement of dopamine release from cytoplasmic pool, and inhibition of dopamine reuptake by MAP, are involved in MAPs acute behavioral effects. Further, the fact that neither TBZ administration following GBR-12909 pretreatment, nor oxypertine treatment following MAP pretreatment, elicited transient hyperactivity suggests that dopamine is involved in hyperactivity elicited by post-MAP treatment with TBZ. It is also suggested that inhibition of monoamine oxidase (MAO) by MAP and dopamine displacement by TBZ may be responsible for the transient stimulation produced by 3–6 h post-MAP treatment with TBZ. It is hypothesized that the MAO inhibitory action of MAP persists after cessation of its acute stimulant effect, possibly up to 6 h after administration.

Methamphetamine Tetrabenazine Monoamine depletion Monoamine oxidase inhibition
Ambulation Behavioral stimulation Anti-methamphetamine effect Mice

THE CNS stimulant effect of amphetamines is caused by their facilitation of catecholaminergic (mainly dopaminergic) neurotransmission. Thus, amphetamines enhance catecholamine release from the cytoplasmic (releasable) pool and inhibit uptake of catecholamine at presynaptic nerve terminals (6). In addition to such actions, it is also suggested that amphetamines have inhibitory action on monoamine oxidase (MAO) (4,8). Since the role of MAO inhibition in psychopharmacological effects of the amphetamines is thought to be less pronounced than amphetamine-induced catecholamine release and reuptake blockade behavioral studies on MAO inhibition induced by amphetamines has not been conducted.

Administration of a MAO-inhibitor (e.g., nialamide) alone to rodents induces little behavioral excitation. However, when rodents have been pretreated with a MAO-inhibitor, the administration of tetrabenazine (TBZ) (7), a drug which displaces monoamines from the "cytoplasmic pool," is followed by a transient behavioral excitation (1). Such behavioral excitation can be explained as follows: In absence of a MAO-inhibitor, displaced dopamine from the cytoplasmic pool was rapidly oxidized by MAO, thus preventing behavioral excitation. In this case TBZ-induced depletion of pooled dopamine results in neuroleptic effect (including sedative and anti-amphetamine effects). In contrast, in the presence of a MAO-

inhibitor, the oxidation of released dopamine is delayed, resulting in increased levels of synaptic dopamine, and transient behavioral excitation.

According the abovementioned interactions between TBZ and MAO-inhibitors, and the monoamine-depleting properties of TBZ, the followings can be hypothesized to result from the interaction between MAP and TBZ:

1. Pretreatment with TBZ will inhibit the stimulant effect of amphetamines due to depletion of catecholamines from the cytoplasmic pool;
2. If amphetamines have an inhibitory action on MAO, post-amphetamine treatment with TBZ will be followed by behavioral excitation due to the catecholamine displacement by TBZ, and the delay of the oxidation of released catecholamines by MAO-inhibition induced by amphetamines.

To test these predictions, modification of the behavioral stimulant effect of MAP by pretreatment or posttreatment with TBZ was evaluated in terms of ambulation in mice.

METHOD

Animals

Male mice of the dd strain (Institute of Experimental Animal Research, Gunma University School of Medicine, Maebashi, Japan) were used at 6 weeks of age and at a weight of 25–28 g. Groups of 10 mice each were housed in polycarbonate cages (25W × 15D × 15H cm), and were freely given free access to a solid diet (MF; Oriental Yeast, Tokyo, Japan) and tap water. Conditions of the breeding room were well controlled (temperature; 23°C ± 1°C, relative humidity; 55 ± 3%, and a 14L : 10D cycle; lights on at 0500–1900 h).

All experiments were conducted according to The Japanese Guidelines for the Care and Use of Laboratory Animals.

Apparatus

Ambulation of mice was measured with a tilting-type ambulometer having 10 bucket-like Plexiglas activity cages of 20 cm in diameter (SMA-10: O'Hara & Co., Tokyo, Japan). This ambulometer recorded horizontal movements (ambulation), but not any vertical movements, of mice.

Drugs

The drugs used were methamphetamine HCl (MAP; Dainippon Pharm., Osaka, Japan), tetrabenazine HCl (TBZ; Pfizer Taito, Tokyo, Japan), oxypertine (Daiichi Pharm., Tokyo), GBR-12909 (Nippon Chemiphar, Tokyo), and nialamide HCl (ICN Pharm., Amsterdam). These drugs except for oxypertine were dissolved or in physiological saline, and administered subcutaneously. Oxypertine was suspended in physiological saline, and administered intraperitoneally. The volume administered was fixed to 0.1 ml/10 g body weight of the mouse. The dose of MAP was constant at 2 mg/kg, which was considered to be optimum for increasing the ambulation of mice without eliciting strong stereotypy (3).

Experimental Procedures

Groups of 10 mice each were used in all experiments mentioned below. The drug treatments and behavioral tests were carried out between 0900–1600 h.

Experiment 1. Administrations of TBZ, and then MAP. Four sets of three groups of mice were pretreated with saline or TBZ (4 mg/kg), and then given saline or MAP (2 mg/kg) at 2, 1, or 0.5 h after the pretreatment. In addition, another two groups of mice were pretreated with TBZ (1 and 2 mg/kg), and then given MAP at 0.5 h after the pretreatment. Ambulation of mice was observed for 0.5 h before and after the administration of saline or MAP.

Experiment 2. Administrations of MAP, and then TBZ. After adaptation of mice to the activity cage for 0.5 h four groups of mice were given either saline alone, TBZ (4 mg/kg) alone, MAP (2 mg/kg) alone or the combination of MAP (2 mg/kg) with TBZ (4 mg/kg), and their ambulation was measured for 0.5 h. Other two groups of mice were given a combination of MAP (2 mg/kg) with TBZ (1 and 2 mg/kg).

Furthermore, four sets of eight groups of mice were given saline or MAP (2 mg/kg), and were placed in the activity cages. These groups were then treated with saline or TBZ (4 mg/kg) at either 0.5, 1, 2, 3, 4, 5, 6, or 7 h after the saline or MAP pretreatment. The other two groups of mice were given MAP (2 mg/kg), and then 4-h post-MAP treatment with TBZ (1 and 2 mg/kg). Ambulation was measured for 0.5 h after the second treatment.

Experiment 3. The other drug combinations. Three sets of two groups of mice were pretreated with MAP (2 mg/kg), nialamide (20 mg/kg) or GBR-12909 (10 mg/kg). Four hours after such pretreatments the following trearments were conducted: Saline or oxypertine (10 mg/kg) to MAP pretreated groups, saline or TBZ (4 mg/kg), and saline or TBZ (4 mg/kg) to GBR-12909 pretreated groups.

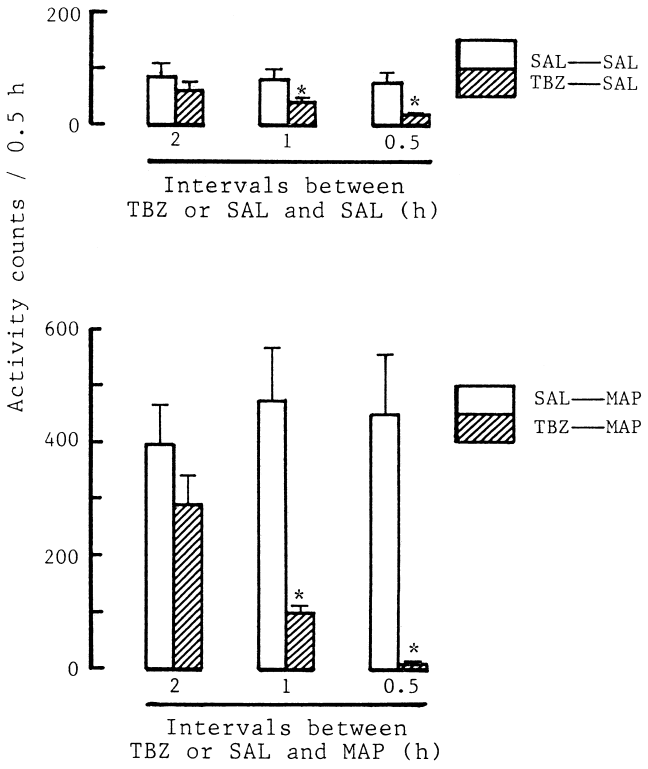

FIG. 1. Mean 0.5-h ambulatory activity counts (with SEM) after subcutaneously administration of saline (SAL) (upper panel) or methamphetamine (MAP: 2 mg/kg) (lower panel) to the mice pretreated with saline or tetrabenazine (TBZ: 4 mg/kg SC) at 2, 1, or 0.5 h before the administration of saline or methamphetamine. $p < 0.05$ vs. the saline-pretreated group. N = 10 in each group.

Statistical Analysis

The mean overall ambulatory activity counts for 0.5 h following the administration of saline or MAP (Experiment 1), or posttreatment with saline or TBZ (Experiment 2), were analyzed by two- or three-way analysis of variance (ANOVA). The factors were TBZ doses (2 levels including MAP alone or saline as dose = 0), MAP doses (2 levels including TBZ alone or saline as dose = 0), and time-points (intervals separating the treatments: 3 levels in Experiment 1, and 9 levels in Experiment 2 including combined administration of two drugs as interval = 0). Further, one-way ANOVA was applied for assessment of the dose-dependency of TBZ effect (4 levels including saline as dose = 0). Posthoc analyses were conducted by paired *t*-test or Dunnett's test. In Experiment 3, paired *t*-test was conducted. Values of p less than 0.05 were considered statistically significant.

RESULTS

Experiment 1

The ambulatory activity following administration of saline or MAP in mice pretreated with saline or TBZ was significantly influenced by MAP treatment [$F(1,108) = 315.8$, $p < 0.001$], TBZ pretreatment [$F(1,108) = 89.2$, $p < 0.001$] and the interval separating the treatments [$F(2,108) = 63.9$, $p < 0.001$]. There were significant pretreatment X time-point [$F(2,108) = 17.1$, $p < 0.001$] and pretreatment X treatment X time-point [$F(2,108) = 6.0$, $p < 0.001$] interactions. However, treatment X time-point [$F(2,108) = 1.5$, ns] and pretreatment X treatment [$F(1,108) = 0.9$, ns] interactions were not significant. As shown in upper panel of Figure 1, even though the pretreatment with saline or TBZ followed by administration of saline elicited very low activity counts, 1- and 0.5-h pretreatment with TBZ further decreased activity counts as compared with saline pretreatment. As shown in lower panel of Figure 1, the activity counts following administration of MAP were higher than those after administration of saline (see upper panel of Fig. 1). Pretreatments with TBZ (4 mg/kg) at 1 and 0.5 h significantly inhibited the ambulation-increasing effect of MAP. Particularly, 0.5-h pretreatment with TBZ completely inhibited MAP effect. Pretreatment with saline did not alter the effect of MAP on ambulation.

The ambulation-increasing effect of MAP was significantly inhibited by 0.5-h pretreatment with TBZ [$F(3,36) = 46.7$, $p < 0.001$]. Posthoc analyses revealed that, as compared with saline pretreatment, the activity counts were significantly lower in the groups pretreated with 1–4 mg/kg TBZ.

Experiment 2

The ambulatory activity associated with post-MAP (or saline) treatment with saline or TBZ was significantly dependent on MAP pretreatment [$F(1,324) = 509.3$, $p < 0.001$], TBZ treatment [$F(1,324) = 75.0$, $p < 0.001$] and the interval separating the treatments [$F(8,324) = 83.9$, $p < 0.001$]. There were significant MAP pretreatment X TBZ treatment effects [$F(1,324) = 43.8$, $p < 0.001$], MAP pretreatment X time-point interactions [$F(8,324) = 92.7$, $p < 0.001$], TBZ treatment X time-point interactions [$F(8,324) = 12.7$, $p < 0.001$], and MAP pretreatment X TBZ treatment X time-point interactions [$F(8,324) = 8.3$, $p < 0.001$]. As shown in upper panel of Fig. 3, postsaline treatment with saline or TBZ did not alter ambulatory activity, and the activity counts were always less than 100. When compared with the activity counts following administration of MAP alone, combined treatment with MAP and TBZ (at the "0 h" time-point) yielded increased activity score. There was no significant effect of post-MAP TBZ treatment at 0.5–2 h. However, post-MAP treatment with TBZ at

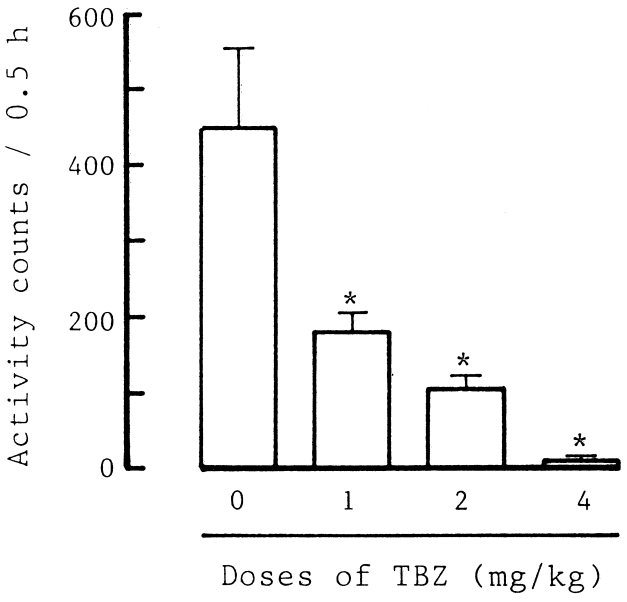

FIG. 2. Mean 0.5-h ambulatory activity counts (with SEM) after SC administration of methamphetamine (2 mg/kg) to the mice pretreated with tetrabenazine (TBZ: 1, 2, and 4 mg/kg SC) or saline (tetrabenazine-dose = 0) at 0.5 h before the administration of methamphetamine. $p < 0.05$ vs. the saline-pretreated group. N = 10 in each group.

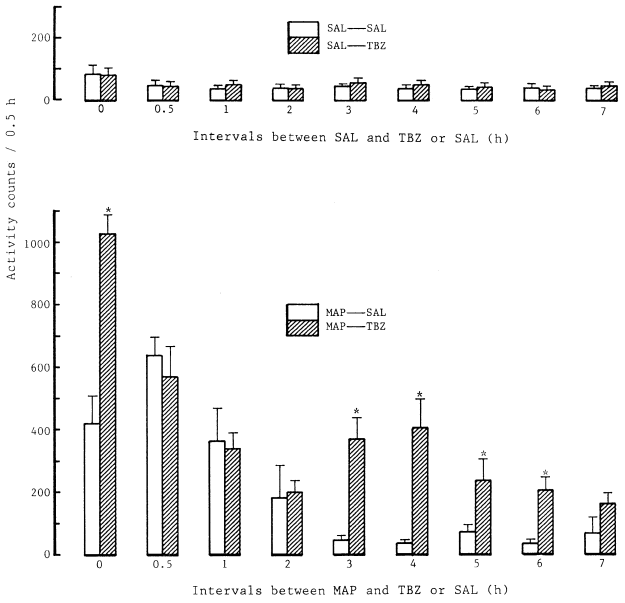

FIG. 3. Mean 0.5-h ambulatory activity counts (with SEM) following 0–7 h postsaline (SAL) treatment with saline or tetrabenazine (4 mg/kg) (upper panel), and 0–7 h postmethamphetamine (MAP: 2 mg/kg) treatment with saline or tetrabenazine (lower panel). $p < 0.05$ vs. the group given methamphetamine alone (interval N = 0) or postmethamphetamine treatment with saline. N = 10 in each group.

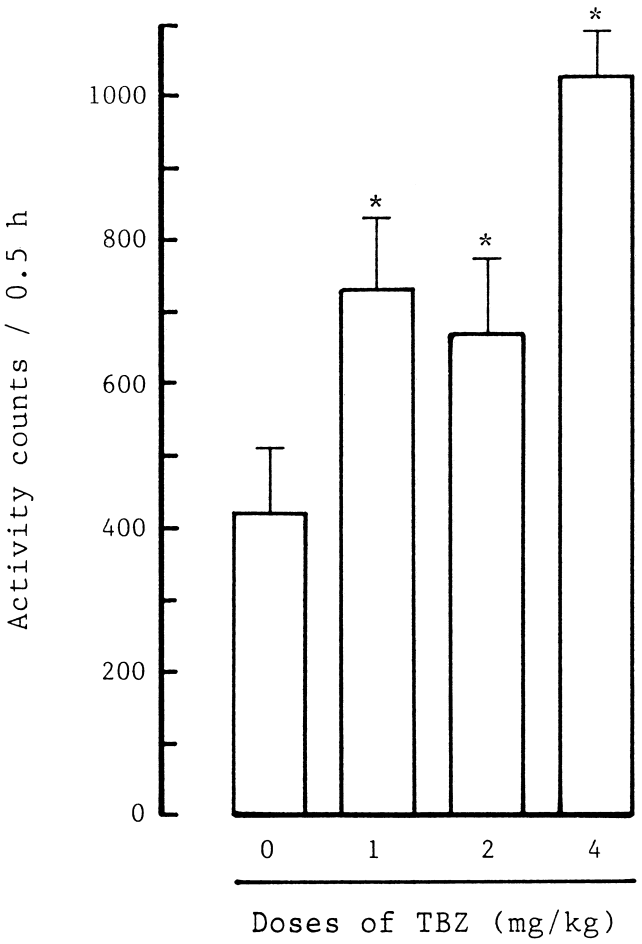

FIG. 4. Mean 0.5-h ambulatory activity counts (with SEM) immediately after SC administration of methamphetamine (MAP: 2 mg/kg) alone (dose of tetrabenazine (TBZ = 0), or MAP in combination with tetrabenazine (1, 2, and 4 mg/kg). $p < 0.05$ vs. the group given methamphetamine alone. N = 10 in each group.

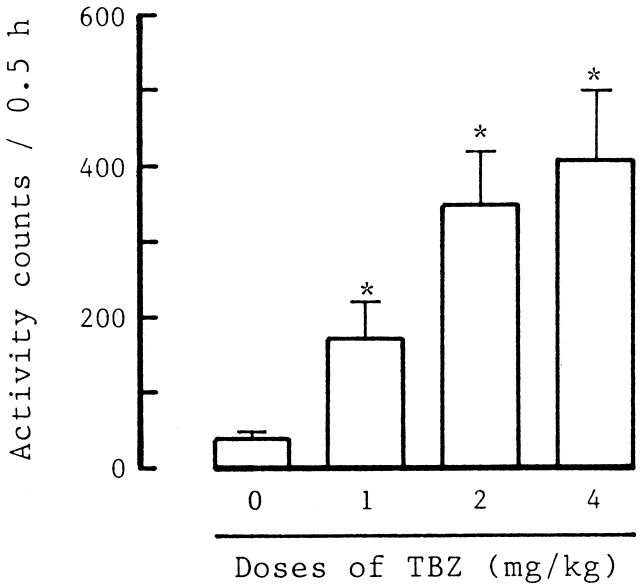

FIG. 5. Mean 0.5-h ambulatory activity counts (with SEM) immediately after the administration of tetrabenazine (TBZ: 0; saline, 1, 2, and 4 mg/kg) to the mice given methamphetamine (2 mg/kg SC) at 4 h before the administration. $p < 0.05$ vs. the group given saline. N = 10 in each group.

3–6 h, but not at 7 h, was followed by a transient (0.25–0.5 h) increase in the ambulatory activity. Post-MAP treatment with saline at these time-points was never followed by increase in ambulation.

The enhancement of ambulation-increasing effect and the transient increase in the ambulation caused by 0-h and 4-h post-MAP treatments with TBZ, respectively, were dependent on TBZ-dose [$F(3,36) = 12.8$ and 20.3, respectively, $p < 0.001$]. Thus, as shown in Figs. 4 and 5, the activity counts following combines treatment with MAP and TBZ (1–4 mg/kg), and those 4-h post-MAP treatment with TBZ (1–4 mg/kg) were significantly greater than the counts following MAP alone and post-MAP treatment with saline, respectively.

Experiment 3

The results are presented in Table 1. GBR-12909 (10 mg/kg) induced hyperactivity in mice with almost the same potency and time course as those proced by MAP (2 mg/kg). Thus, the activity of mice returned to the predrugged level by 3 h after the dministration of GBR-12909. Nialamide did not cause any increase in the ambulation. Neither post-MAP treatment with oxypertine nor post GBR-12909 treatment with TBZ did not produce transient increase in ambulation. In contrast, postnialamide treatment with TBZ was followed by prominent increase in the ambulation.

DISCUSSION

The ambulation-increasing effect of subcutaneously administered MAP reaches its peak at 0.5–1 h, and persists for approximately 3 h (e.g., ref. 3). The activity-enhancing effect of MAP followed by 0.5–7 h post-MAP treatment with saline (Fig. 3, lower panel) was similar to the time course of change in the activity obtained by continuous measurement after the administration of MAP (3), indicating that the cues associated with saline injection did not alter the effect of MAP.

It has been reported that MAP elicits CNS stimulant effect by accelerating catecholamine (particularly dopamine) release from the cytoplasmic interneural pool, and inhibiting catecholamine reuptake at presynaptic terminals (6). In contrast, TBZ displays a "neuroleptic-like" profile by depleting catecholamines from the cytoplasmic pool (7). Such neurochemical properties of MAP and TBZ are supported by the present findings that the ambulation-increasing effect of MAP was significantly inhibited by 1–0.5 h pretreatments with TBZ. Thus, at the time of MAP administration, releasable catecholamine from the cytoplasmic pool had already been depleted by pretreatment with TBZ. Nevertheless, 2-h pretreatment with TBZ did not inhibit the effect of MAP. This result may be caused by recovery of the cytoplasmic catecholamine pool within 2 h after the administration of TBZ. The duration of anti-MAP action of TBZ (presumably, significant depletion of the cytoplasmic catecholamine pool) is similar to the time course of its anti-avoidance action (5).

Displacement of catecholamine from the cytoplasmic pool by TBZ may transiently increase the concentration of synaptic catecholamine. However, the administration of TBZ alone,

TABLE 1

AMBULATORY ACTIVITY COUNTS (MEAN ± S.E.M.) FOR 0.5 H AFTER ADMINISTRATION OF DRUGS TO MICE*

Drugs**		
First	Second	Activity count
Methamphetamine (2 mg/kg SC)	Saline (IP)	42 ± 19
Methamphetamine (2 mg/kg SC)	Oxypertine (2 mg/kg IP)	35 ± 12
Nialamide (20 mg/kg SC)	Saline (SC)	15 ± 6
Nialamide (20 mg/kg SC)	Tetrabenazine (4 mg/kg SC)	389 ± 74$
GRB-12909 (10 mg/kg IP)	Saline (SC)	86 ± 29
GRB-12909 (10 mg/kg IP)	Tetrabenazine (4 mg/kg SC)	53 ± 16

*: Activity counts following the second drug administration are presented. **: The second drug administration was carried out 4 h after the first drug adminsitration. $: $p < 0.001$ vs. the control group given saline in the second administration. $N = 10$ in each group.

or postsaline treatment with TBZ, never increased the ambulatory activity of mice. Since the released catecholamine is rapidly metabolized by MAO, it could be explained that the amount of displaced catecholamine remaining unmetabolized by MAO may not have been sufficient to elicit hyperactivity. In contrast, the remarkable enhancement of MAPs stimulant effect produced by combined administration of MAP with TBZ might be due to an additive effect of catecholamine release induced by MAP (6) and catecholamine displacement induced by TBZ (7). The fact that the enhancement of ambulation-increasing effect of MAP was dependent on the doses of TBZ may support this consideration.

Post-MAP treatments with TBZ at 0.5–2 h scarcely modified the stimulant effect of MAP. Since MAP provokes considerable catecholamine release and behavioral excitation on its own, such actions of MAP may sufficient to mask the effect of TBZ-induced catecholamine displacement.

The most interesting result demonstrated in this study was a transient (0.25–0.5 h), but a significant, increase in ambulatory activity induced by 3–6 h post-MAP treatment with TBZ. In contrast, the 3–6 h post-MAP treatment with saline was never followed by a significant increase in ambulation. As mentioned above, the acute stimulant effect of MAP had already disappeared at those time-points. The preliminary study also revealed that, when mice were pretreated with nialamide, a MAO-inhibitor, the administration of TBZ was followed by a marked increase in ambulation for 0.66–0.5 h. These findings suggest the possibility that the transient ambulatory stimulation caused by post-MAP treatment with TBZ was due to an interaction between the MAO-inhibitory action of MAP and catecholamine displacement from cytoplasmic pool by TBZ. Neurochemical analyses were not carried out in this study. However, preliminary experiments revealed that neither post-MAP treatment with oxypertine (a selective norepinephrine releaser/depleter), nor administration of GBR-12909 (a dopamine reuptake inhibitor) followed by TBZ resulted in transient behavioral excitation similar to that induced by post-MAP or postnialamide treatment with TBZ. These preliminary data support the consideration that dopamine, rather than norepinephrine, is involved in the interaction between MAP and TBZ, and that MAO-inhibition is mainly involved in the transient excitation following post-MAP treatment with TBZ. The present interaction between MAP and TBZ suggests that MAO-inhibitory action of MAP reaches a peak at approximately 4 h, and persists for 6 h after administration. The acute stimulant effect of MAP disappears within 3 h. However, such a long-lasting property of MAO-inhibitory action of MAP is consisted with the report that MAP is detectable up to 10 h after administration in rats (2).

In summary, the following may be derived from the present results:

1. Pretreatment with TBZ depletes dopamine from the cytoplasmic pool, and this action results in inhibition of the CNS stimulant effect of MAP.
2. It is reasonable to hypothesize that the transient behavioral excitation observed when TBZ is administered after MAP pretreatment is resulted from the delay of the oxidation of TBZ-displaced dopamine. This delay may be due to MAP-induced MAO inhibition.
3. The presumed MAO-inhibitory effect of MAP persists for 6 h after administration, although its acute stimulant effect disappears with 3 h.

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